III. Sequence Rules

The Examiner states that claims 9 and 11 contain sequences that are not identified by SEQ ID NO and that it appears as though each of the sequences should be labeled SEQ ID NO: 1-5 respectively. Claims 9 and 11 have been amended to identify the recited sequences by SEQ ID NO.

IV. Claim Objections

Claims 1, 9 and 11 have been objected to because they do not end in a period. These claims have been amended accordingly. Reconsideration and withdrawal of the objection are respectfully requested.

V. Information Disclosure Statement

An Information Disclosure Statement (IDS) is being filed concurrently herewith, with the appropriate fee. Entry of the IDS is respectfully requested.

VI. Claim Rejections – 35 U.S.C. § 112 – Second Paragraph

Claims 1-19 have been rejected under 35 U.S.C. § 112 second paragraph as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

The Examiner states that claims 1-19 are indefinite because claim 1 does not contain a final process step. Applicant has amended claim 1 to contain a final process step which relates back to the preamble as suggested by the Examiner. Claim 1 has also been amended to recite proper antecedent basis.

The Examiner states that claims 3-6 are indefinite over the recitation "at least about" because the metes and bounds of the invention are not clear. Applicant has amended claims 3, 4 and 5 to recite "at least 10 nucleotides," "at least 35 nucleotides," and "at least 25 nucleotides" respectively. Applicant has amended claim 6 to recite "from about 15 to about 20 nucleotides."

The Examiner states that claims 9 and 11 are indefinite because the claims do not refer to the sequences in the alternative. Claims 9 and 11 have been amended to recite proper Markush language.

The Examiner states that claim 19 is indefinite because it is unclear what a "method according to claim 1 substantially as herein before described" encompasses. Applicant has cancelled claim 19, thereby obviating the rejection.

In view of the above amendments to the claims, Applicant respectfully requests reconsideration and withdrawal of the rejection.

VII. Rejection of Claim 18 under 35 U.S.C. § 102(e)

Reasons for the Rejection

Claim 18 is rejected under U.S.C. § 102(e) as being anticipated by Tyagi, et al., U.S. Patent 6,037,130, (hereinafter "Tyagi '130"). The Examiner states that the intended use of the product for distinguishing methylated and non-methylated nucleic acids does not impart any structural limitations which are not taught by Tyagi '130. The Examiner also states that Tyagi '130 teaches a kit comprising a detector probe which is a fluorescently labeled hairpin forming oligonucleotides containing a fluorescent emitter and a quencher. Applicant respectfully traverses the rejection to the extent it is maintained over the claims as amended.

Response to the Rejection

Claim 18 has been amended to incorporate the oligonucleotide sequence limitations of claim 1. The present invention is directed to a method for detecting methylated nucleic acids comprising the step of contacting a nucleic acid with an oligonucleotide sequence. The oligonucleotide sequence comprises the following two labels:

a) a fluorophore; and
b) a quencher.

In contrast, Tyagi '130 teaches a fluorescently labeled hairpin forming oligonucleotide containing three moieties:

a) a fluorescent emitter moiety;
b) a fluorescent harvester moiety; and
c) a quencher moiety.

A fluorophore is defined by the present specification as a "chemical compound which when excited by exposure to particular wavelengths of light, emits light (i.e., fluoresces) at a different wavelength" (See page 13, lines 1-3). While both the present invention and Tyagi '130 describe an oligonucleotide having a quencher, the present invention and Tyagi '130 differ because the present invention describes an oligonucleotide having one fluorophore, while Tyagi '130 requires an oligonucleotide having an additional second fluorophore. Tyagi '130 requires a first fluorophore to serve as an emitter, and requires a second fluorophore to serve as a harvester.

Claim 18 as amended herein requires a loop sequence, which is complementary to a nucleic acid sample region, where the nucleic acid sample region is susceptible to methylation. The present claims are patentably distinguishable from Tyagi '130. Tyagi '130 does not teach an oligonucleotide having only a single fluorophore. Furthermore, Tyagi '130 does not teach an oligonucleotide loop sequence that is complimentary to a sample sequence wherein the sample sequence is susceptible to methylation.

The present invention is an improvement over Tyagi '130 in that only one fluorophore is needed. Furthermore, the present invention is an improvement over Tyagi '130 in that it can have a probe containing an oligonucleotide loop sequence that is complimentary to a sample sequence wherein the sample sequence is susceptible to methylation. Applicant respectfully requests that the rejection under 35 U.S.C. § 102(e) be reconsidered and withdrawn.

VIII. Rejection under 35 U.S.C. § 103(a)

A) Rejection of Claims 1-6, 14-17 and 19 under 35 U.S.C. § 103(a)

Reasons for Rejection

Claims 1-6, 14-17 and 19 are rejected under 35 U.S.C. § 103(a) over Elsas, et al., U.S. Patent 6,207,387 (hereinafter "Elsas") in view of either Ehrlich, et al., *Biochimica et Biophysica Acta*, Vol. 395, pages 109-119, 1975 (hereinafter "Ehrlich") or Hua, et al., *Gov. Rep. Announce. Index US*, Vol. 88, No. 18, Abstract No. 847,050 1988 (hereinafter "Hua") and in further view of either Tyagi, et al., U.S. Patent 6,150,097 (hereinafter "Tyagi '097") or Coull, et al., U.S. Patent 6,355,421 (hereinafter "Coull").

The Examiner states that it would have been obvious to modify the method of Elsas for detecting different nucleic acids, with 1) the teachings of either Tyagi '097 or Coull which discuss stem-loop and fluorescence energy transfer, and 2) the teachings of Ehrlich or Hua which discuss the properties of methylated DNA. Applicant respectfully disagrees with the grounds of the rejection.

Response to the Rejection

Elsas is drawn to a process for detecting "missense" or "nonsense" mutations in the gene responsible for galactosemia. The mutations described by Elsas are nucleic acid substitutions. Elsas does not teach or suggest mutations wherein the nucleic acid is methylated. Furthermore, Elsas does not teach or suggest a process for detecting methylation of a gene. The process of Elsas uses a probe having a fluorescent label. Elsas does not teach or suggest using a probe having both a fluorescent label and a quencher. The process of Elsas uses the temperature of hybridization between the probe and the gene to discriminate between an exact sequence match and a mismatch. As stated by the examiner, "Elsas does not specifically teach the structure of fluorescence energy transfer and does not teach using the fluorescence energy transfer for detecting methylation." Elsas also does not teach or suggest that the temperature of hybridization can be used to discriminate between methylated and nonmethylated nucleic acids.

Neither the teaching of Ehrlich or Hua, even in view of Tyagi '097 or Coull are able to remedy the deficiencies of Elsas.

Ehrlich investigated the physical and chemical properties of bacteriophage XP-12, in which all of the cytosine residues are replaced by 5-methylcytosine. Ehrlich reports that methylation of cytosine increases the melting temperature of XP-12 over non-methylated XP-12.

Hua calculates the increase in melting temperature of methylated Z-DNA over unmethylated B-DNA using a modified self-consistent effective photon approximation. Neither Ehrlich or Hua teach or suggest the effects on melting temperature of a helix having one methylated strand and one non-methylated strand. Neither Ehrlich nor Hua teach or suggest a process for using the property of melting temperature for detecting methylated nucleic acid using a non-methylated probe.

Tyagi '097 describes a probe capable of undergoing a conformational change upon interacting with a target in an assay preferably having a fluorophore and a quencher. Tyagi '097 does not teach or suggest the use of probes to detect methylated nucleic acids.

Coull describes protein nucleic acid (PNA) molecular beacons in place of deoxyribonucleic acid (DNA) molecular beacons comprising self-complementary arm segments and flexible linkages that promote intramolecular or intermolecular interactions. Coull does not teach or suggest intermolecular interactions between nucleic acid sequences in which a sequence is susceptible to methylation. Moreover, the nucleic acid sequences described by Coull are PNA sequences rather than DNA sequences. Coull uses the term "melting" referring to the term "hairpin melting" only in conditions of background "noise." Coull does not teach or suggest the detection of methylated nucleic acids. Coull also does not teach or suggest that melting temperature can be used to discriminate methylated and non-methylated nucleic acids.

The Examiner has failed to make out a *prima facie* case of obviousness. The Manual of Patent Examining Procedure (MPEP) states at § 2142 that

> To establish a prima facie case of obviousness, three basic criteria must be met. First, there must be some suggestion or motivation, either in the references themselves or in the knowledge generally available to one of ordinary skill in the art, to modify the reference or to combine reference teachings. Second, there must be a reasonable expectation of success. Finally, the prior art reference (or references when combined) must teach or suggest all the claim limitations. The teaching or suggestion to make the claimed combination and the reasonable expectation of success must both be found in the prior art, and not based on applicant's disclosure.

The Federal Circuit has stated that "[o]bviousness cannot be established by combining the teachings of the prior art to produce the claimed invention, absent some teaching[,] suggestion or incentive supporting the combination" (*In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987)) and that "[i]t is impermissible . . . simply to engage in a hindsight reconstruction of the claimed invention, using the applicant's structure as a template and selecting elements from references to fill the gaps. . . . The references themselves must provide some teaching whereby the applicant's combination would have been obvious." (*In re Gorman*, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991)).

Applicant believes that the invention is being used as a blueprint for hindsight assembly of the cited references. This is impermissible. "Combining prior art references without evidence of such a suggestion, teaching, or motivation simply takes the inventor's disclosure as a blueprint for piecing together the prior art to defeat patentability -- the essence of hindsight." *In re Dembiczak* (50 USPQ2d 1614 (Fed. Cir. 1999)).

Combining the references of Elsas, Ehrlich, Hua, Tyagi '097 and Coull fails to render obvious Applicant's invention. The mere observation by Ehrlich and Hua that melting temperatures of methylated DNA sequences can be higher than melting temperatures of non-methylated DNA sequences is not a teaching or suggestion to modify the method of Elsas from detecting nucleic acid substitutions in a sample to detecting methylated nucleic acids in a sample. Substitution of a cytosine residue with a different nucleotide base is entirely different from substitution of a cytosine nucleotide base with a methylated cytosine base. A person skilled in

the art would not have any reasonable expectation that the two different types of mutations would have the same melting behavior.

The combination of all five references fails to teach or suggest the claimed invention. The Examiner has not presented any reason as to why a person skilled in the art, with all of the references in place, would be in possession of the Applicant's claimed invention. First, the combination of all five references fails to teach or suggest all the claim limitations. None of the references teach the element of detecting methylated nucleic acids, or the element of an oligonucleotide sequence containing a region that is susceptible to methylation. Only in Applicant's disclosure is the element of detecting methylated nucleic acids found. Second, beyond a wish for cancer diagnosis, the Examiner points neither to any specific passages in the cited references which provide motivation to combine those references, nor to any other sources for such motivation of the references to produce Applicant's invention. Third, the Examiner does not provide any reference that teaches or suggests the expectation of success of detecting methylated DNA using the specified combination of references.

By failing to present references that 1) recite every element of the claims, 2) provide motivation for the combining of the specific references cited in the Office Action, and 3) provide reasonable expectation of success, a *prima facie* case of obviousness has not been made. Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

B) Rejection of Claims 7, 10, 12 and 13 under 35 U.S.C. § 103(a)

Reasons for the Rejection

Claims 7, 10, 12 and 13 are rejected under 35 U.S.C. § 103(a) over Elsas, in view of either Ehrlich or Hua and in further view of either Tyagi '097 as applied to Claims 1-6, 14-19 above, and further in view of Herman, et al., U.S. Patent 6,265,171 (hereinafter "Herman").

The Examiner states that it would have been obvious to use the method of Elsas, Ehrlich or Hua and Tyagi '097 or Coull in view of the teaching of Herman in which genes including

GSTpi and calcitonin are differentially methylated at CpG islands in neoplastic versus normal tissue. Applicant respectfully disagrees with the grounds of the rejection.

Response to the Rejection

Herman does not teach or suggest using the combined teachings of Elsas, Ehrlich, Hua, Tyagi '097 or Coull to detect the presence of methylated nucleic acids by the methods of Applicant's claims. Just as Ehrlich, Hua, Tyagi '097 and Coull are not able to remedy the deficiencies of Elsas, Herman is not able to either.

Herman only teaches a method for detecting a methylated CpG-containing nucleic acids present in a specimen. Herman's method includes a first step of modifying DNA by sodium bisulfite or a comparable agent which converts all unmethylated but not methylated cytosines to uracil, and a subsequent second step of amplifying the DNA with primers specific for methylated DNA and not unmethylated DNA. Herman detects methylated nucleic acids based on the presence of amplification products produced in the amplification step. Herman is silent with respect to detecting methylated nucleic acids using oligonucleotide probes. Moreover, Herman is silent regarding any motivation to combine the specific references to achieve Applicant's claimed invention.

Herman does not provide any motivation to combine the particular references cited by the Examiner. The only motivation that Herman provides is the desire to detect methylation of CpG because the presence of methylated CpG in a 5' regulatory region can be indicative of a cell proliferative disorder such as various types of cancer.

A motivation to achieve a goal is not the same thing as a motivation to combine particular references to achieve that goal. The Examiner may have cited a reference that provided the motivation to achieve the goal of detecting methylated nucleic acids. However, the Examiner has not cited any reference that provides the necessary motivation to choose the particular cited references from all possible references, and to combine them in a way that arrives at Applicant's specific method of detecting methylated nucleic acids. The Examiner has therefore failed to establish a prima facie case of obviousness.

Claims 7, 10, 12 and 13 depend from independent Claim 1. Because the combination of the references fails to render independent claim 1 obvious, the combination fails to render all dependent claims as obvious as well. Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

C) Rejection of Claims 7 and 8 under 35 U.S.C. § 103(a)

Reasons for the Rejection

Claims 7 and 8 are rejected under 35 U.S.C. § 103(a) over Elsas in view of either Ehrlich or Hua and in further view of either Tyagi '097 or Coull as applied to Claims 1-6, 14-19 above, and further in view of Kay, et al., *Leukemia and Lymphoma*, Vol. 24, pages 211-220, 1977 (hereinafter "Kay").

The Examiner states that it would have been obvious to use the method of Elsas, Ehrich or Hua and Tyagi '097 or Coull, in view of the teaching of Kay, in which the Myf-3 gene is abnormally hypermethylated. Applicant respectfully disagrees with the grounds of the rejection.

Response to the Rejection

Kay does not teach or suggest using the combined teachings of Elsas, Ehrlich, Hua, Tyagi '097 or Coull to detect the presence of methylated nucleic acids. Just as Ehrlich, Hua, Tyagi '097 and Coull are not able to remedy the deficiencies of Elsas, Kay is not able to either.

Kay only teaches that the Myf-3 gene can be hypermethylated and that the hypermethylated status of the Myf-3 gene may provide new diagnostic indicators of malignancy. Kay is silent with respect to detecting methylated nucleic acids using oligonucleotide probes. Moreover, Kay is silent regarding any motivation to combine the particular references cited by the Examiner to achieve Applicant's claimed invention. The only motivation that Kay provides is the desire for more extensive studies in the diagnosis of malignant lymphomas.

A motivation to achieve a goal is not the same thing as a motivation to combine particular references to achieve that goal. The Examiner may have cited a reference that provided the

motivation to achieve the goal of detecting methylated nucleic acids. However, the Examiner has not cited any reference that provides the necessary motivation to choose the particular cited references from all possible references, and to combine them in a way that arrives at Applicant's specific method of detecting methylated nucleic acids. The Examiner has therefore failed to establish a prima facie case of obviousness.

Claims 7 and 8 depend from independent Claim 1. Because the combination of the references fails to render independent claim 1 obvious, the combination fails to render all dependent claims as obvious as well. Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

Applicant submits that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicant respectfully requests the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend the claims as follows:

1. (Amended) A method for detecting methylated nucleic acids comprising the steps of:

(i) contacting a nucleic acid sample suspected of containing methylated nucleotides with an oligonucleotide [sequence] probe under suitable conditions for nucleic acid hybridization, said oligonucleotide [sequence] probe characterized in that,

(a) it comprises a first stem labeled with a fluorophore moiety, a loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation, and a second stem labeled with a quencher moiety that is capable of quenching the fluorophore moiety when in spatial proximity to the fluorophore moiety; and

(b) the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the probe is dissociated from the nucleic acid sample;

(ii) altering the hybridization conditions such that the oligonucleotide probe dissociates from unmethylated [DNA] nucleic acids but remains hybridized to methylated [DNA] nucleic acids; and

(iii) measuring the change in fluorescence;

wherein an increase in fluorescence indicates methylated nucleotides in said nucleic acid sample.

2. (Amended) A method according to claim 1 wherein the [labeled] oligonucleotide [sequences] probe dissociates from the target nucleic acid sample according to step (ii) the first and second stem hybridi[s]ze together causing quenching of the fluorophore moiety.

3. (Amended) A method according to claim 1 wherein the loop sequence contains at least [about] 10 nucleotides.

4. (Amended) A method according to claim 1 wherein the loop sequence contains at least [about up to] 35 nucleotides.

5. (Amended) A method according to claim 1 wherein the loop sequence contains at least [about] 25 nucleotides.

6. (Amended) A method according to claim 1 wherein the loop sequence contains [at least] from about [from] 15[-] to about 20 nucleotides.

9. (Amended) A method according to claim 8 wherein the [labelled oligonucleotide] loop sequence is complementary to at least one of the sequences selected form the group consisting of:

(i) 5' GCG GCG ACT CCG ACG CGT CCA GCC CGC GCT CC 3' (SEQ ID NO: 1);

(ii) 5' TTA TAC CGC AGG CGG GCG AGC CGC GGG CGC TCG CT 3' (SEQ ID NO: 2); and

(iii) 5' CCG AGA GCC CTG CGG GGC CCG CCC TCC TGC TGG CG 3' (SEQ ID NO: 3).

11. (Amended) A method according to claim 10 wherein the [labelled oligonucleotide] loop sequence is complementary to at least one of the sequences selected from the group consisting of:

(i) 5' CTC CAG CGA AGG CCT CGC GGC CTC CGA GCC TTA TAA G 3' (SEQ ID NO: 4); and

(ii) 5' GGG GAC GCG GGC CGC GCG TAC TCA CTG GTG GCG A 3' (SEQ ID NO: 5).

18. (Amended) A kit for distinguishing methylated and non-methylated nucleic acid sequences, comprising a labeled oligonucleotide [sequence as described herein, which is adapted to distinguish methylated and non-methylated nucleic acid sequences when used in the method according to claim 1] probe; said labeled oligonucleotide probe characterized in that:

(a) it comprises a first stem labeled with a fluorophore moiety, a loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation, and a second stem labeled with a quencher moiety that is capable of quenching the fluorophore moiety when in spatial proximity to the fluorophore moiety; and

(b) the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the oligonucleotide sequence is dissociated from the nucleic acid sample.